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Gastric *Helicobacter* Infection Inhibits Development of Oral Tolerance to Food Antigens in Mice

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The increase in the transcellular passage of intact antigens across the digestive epithelium infected with *Helicobacter pylori* may interfere with the regulation of mucosal immune responses. The aim of this work was to study the capacity of *Helicobacter* infection to inhibit the development of oral tolerance or to promote allergic sensitization and the capacity of a gastro-protective agent, rebamipide, to interfere with these processes in mice. Oral tolerance to ovalbumin (OVA) was studied in 48 C3H/He 4-week-old mice divided into four groups: (i) OVA-sensitized mice; (ii) OVA-"tolerized" mice (that is, mice that were rendered immunologically tolerant); (iii) *H. felis*-infected, OVA-tolerized mice; (iv) and *H. felis*-infected, OVA-tolerized, rebamipide-treated mice. Oral sensitization to hen egg lysozyme (HEL) was studied in 48 mice divided into four groups: (i) controls; (ii) HEL-sensitized mice; (iii) *H. felis*-infected, HEL-sensitized mice; and (iv) *H. felis*-infected, HEL-sensitized, rebamipide-treated mice. Specific anti-OVA or anti-HEL immunoglobulin E (IgE) and IgG1/IgG2a serum titers were measured by enzyme-linked immunosorbent assay. Additionally, the capacity of rebamipide to interfere with antigen presentation and T-cell activation in vitro, as well as absorption of rebamipide across the epithelial monolayer, was tested. *H. felis* infection led to the inhibition of oral tolerance to OVA, but rebamipide prevented this inhibitive effect of *H. felis*. *H. felis* infection did not enhance the sensitization to HEL, but rebamipide inhibited the development of this sensitization. Moreover, rebamipide inhibited in a dose-dependent manner antigen presentation and T-cell activation in vitro and was shown to be able to cross the epithelium at a concentration capable of inducing this inhibitory effect. We conclude that *H. felis* can inhibit the development of oral tolerance to OVA in mice and that this inhibition is prevented by rebamipide.

Infection with *Helicobacter pylori* is very common and is recognized as the main etiopathogenic factor of chronic gastritis and peptic ulcer disease. If not treated, it is a lifelong infection whose implication in extra-digestive disease is suggested although not proven. Data coming from follow-up studies show that, after *H. pylori* eradication, in a subset of patients, chronic gastritis persists for months or even years (19, 39), without a satisfactory explanation for this phenomenon. On the other hand, some data suggest a positive association between *H. pylori* infection and the development of food allergy (8, 16) and other allergic manifestations (31, 35) in humans. We have previously shown that *H. pylori* increases absorption of antigens across the digestive epithelium in vitro (29) and also across the gastric mucosa in vivo in mice (28) and in humans (T. Matysiak-Budnik et al., submitted for publication). In *H. pylori*-infected subjects, this situation may lead to a sustained increased load of food proteins in the digestive mucosa, which could maintain a chronic inflammation and favor food protein sensitization and food allergy in susceptible individuals. Indeed, although the stomach plays an important role as a barrier to the high antigenic load present in the digestive lumen, it is also able to absorb, degrade, and transport macromolecules (10) and can be a target organ for immunoglobulin E (IgE)-mediated reactions to food proteins (5).

Oral administration of soluble antigens leads to a systemic unresponsiveness (i.e., lack of specific antibody production) to the same antigens subsequently delivered systemically, a phenomenon named oral tolerance. In some conditions, oral tolerance can be inhibited, and this can be considered equivalent to promotion of sensitization. Cholera toxin (CT) (13) and *Escherichia coli* heat-labile enterotoxin (7) have been shown to exert such an inhibitory effect. Oral tolerance can be studied by using different murine models (18, 34). C3H/He mice have been used as an experimental model for oral tolerance to ovalbumin (OVA) (18). Moreover, these mice are known to be easily colonized by *H. felis* and to develop gastric inflammation in response to this colonization [M. Maehler, C. Janke, H. J. Hedrich, and S. Wagner, abstract from Digestive Diseases Week of the American Gastroenterological Association, San Diego, Calif., 21 to 24 May 2000, Gastroenterology **118**(Suppl. 2)**:**743, 2000].

Rebamipide is a gastro-protective agent used in the treatment of gastritis (20) and ulcerative colitis, although the mechanisms of its anti-inflammatory action are not completely understood. It reinforces digestive epithelial barrier integrity and inhibits the increased macromolecular transport induced by *Helicobacter* infection in mice (27, 30). These properties could provide protection against allergic sensitization to foreign antigens. Our aim was to study (i) the capacity of *Helicobacter*

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infection to alter the normal and pathological immune responses to ingested antigens, (ii) the capacity of rebamipide to interfere with these processes, and (iii) the possible mechanisms involved in the effect of rebamipide on the immune responses to ingested antigens. Thus, using C3H/He mice as an experimental model, we studied the interference of *Helicobacter* infection and of rebamipide with the development of (i) oral tolerance to OVA and (ii) sensitization to orally administered hen egg lysozyme (HEL) in the presence of CT. *H. felis* has been chosen instead of *H. pylori*, since it is known that mice infected with *H. felis* develop more pronounced gastritis than those infected with *H. pylori* (9). Furthermore, the effect of rebamipide on antigen presentation and T-cell activation in vitro, as well as the in vitro absorption of rebamipide across epithelial intestinal monolayers, was studied.

MATERIALS AND METHODS

Oral tolerance study. Forty-eight 3-week-old female C3H/HeN mice were divided into four groups ($n = 12$). Group I consisted of OVA-sensitized mice which received a single dose of phosphate-buffered saline (PBS) by gastric gavage followed by two subcutaneous injections of OVA (25 and 10 μg) at a 2-week interval. Group II consisted of OVA-"tolerized" mice (that is, mice that were rendered immunologically tolerant) which received a single dose of OVA by gastric gavage (1 mg/g of body weight) followed by two subcutaneous injections of OVA, as described above. Group III consisted of *H. felis*-infected OVA-tolerized mice which were first infected with *H. felis* (100 μl of bacterial suspension [10^9 CFU/ml] introduced by gastric gavage three times at 48-h intervals) and 4 weeks later tolerized to OVA according to the above protocol. Group IV consisted of *H. felis*-infected OVA-tolerized rebamipide-treated mice which were infected with *H. felis* and 4 weeks later tolerized to OVA while receiving additionally a daily treatment with rebamipide (30 μg/day).

All the mice were sacrificed 1 week after the second injection of OVA.

Sensitization study. Four groups of mice were considered in the sensitization study: group I, control mice; group II, HEL-sensitized mice that received HEL (250 μg) by gastric gavage together with CT (10 μg), two times at a 3-week interval; group III, *H. felis*-infected HEL-sensitized mice that were infected with *H. felis* as described for the oral tolerance study and then received two doses of HEL and CT by gastric gavage two times at a 3-week interval; and group IV, *H. felis*-infected HEL-sensitized rebamipide-treated mice that were infected with *H. felis* for 4 weeks and were subsequently sensitized to HEL while receiving a daily oral treatment with rebamipide.

All the mice were sacrificed 3 weeks after the second gavage with HEL.

Measurement of OVA or HEL specific IgE and IgG antibodies. After sacrifice, blood samples were collected by cardiac puncture and plasma was frozen for subsequent measurement of the systemic IgG and IgE antibodies to OVA (tolerance study) or HEL (sensitization study) using an enzyme-linked immunosorbent assay. IgE and total IgG titers were determined as previously described (32).

To detect OVA-specific IgG2a or IgG1 antibodies, the 96-well plates were coated overnight at 4°C with 100 μl of a 10- or 5-μg/ml solution of OVA (ImjectOVA; Pierce) in 0.1 M bicarbonate buffer, respectively. Nonspecific binding sites were blocked with PBS–1% bovine serum albumin (100 μl/well) for 1 h at 37°C. The plates were then incubated with 50-μl aliquots of test sample in PBS-Tween for 2 h at 37°C. Peroxidase-conjugated rat anti-mouse IgG2a and IgG1 (both from PharMingen, Becton Dickinson) were added at 100 μl/well and incubated for 2 h at 37°C. Finally, 100 μl of 3,3′,5,5′-tetramethylbenzidine peroxidase substrate was added to each well. The reaction was stopped with 50 μl of 4 M H_2SO_4/well, and plates were read at 450 nm using an automatic Multiskan microplate reader.

For all antibodies, positive titers were denoted as the last dilution giving an optical density at least two times higher than that of the background.

Measurement of gastric and intestinal anaphylaxis. Stomach and small intestinal fragments were mounted in an Ussing chamber exposing 0.125 cm^2 of surface area, as described previously (28). Potential difference (PD) was checked and electrical resistance (R), an index of epithelial integrity, was calculated. The gastric and intestinal local anaphylactic reactions, reflected by the rise in short-circuit current (Isc) induced by serosally applied OVA or HEL (final concentration, 100 μg/ml), were recorded as previously described (11).

Study of the effect of rebamipide on antigen presentation in vitro. This part of the study was performed in order to test whether the effect of rebamipide on the immune response to dietary antigens in mice could be related to its effect on antigen presentation and T-cell activation. An in vitro model of antigen presentation was used in which human serum albumin (HSA) peptide 64-76 (Syntem, Nimes, France) was used as antigen, a human B-EBV cell line expressing HLA-DR4 molecules (kindly provided by S. Caillat-Zucman) was used as professional antigen-presenting cells (APC), and the HSA-specific HLA-DR4-restricted mouse T-cell hybridoma 17.9 (36) (kindly provided by R. Hershberg), capable of interacting with human antigen-presenting cells in a restricted antigen pathway, was used as effector cells. Rebamipide was used at final concentrations ranging from 0.01 to 2 mM.

B cells were cultured in RPMI 1640 Glutamax (Gibco BRL) containing 10% synthetic serum Prolifix (Bio Media), 1% nonessential amino acids 1% sodium pyruvate (Gibco BRL), and 1% penicillin-streptomycin (Gibco BRL). T-cell hybridoma was cultured in RPMI 1640 Glutamax (Gibco BRL), containing 10% fetal calf serum, 1% sodium pyruvate (Gibco BRL), 1% penicillin-streptomycin (Gibco BRL), and 1% β-mercaptoethanol. For experimental purposes, B cells were diluted to obtain a solution containing 2×10^6 cells/ml, and the T cells were diluted to obtain a solution containing 10^6 cells/ml. B and T cells were incubated separately in a flat-bottom 96-well culture plate (Falcon; Becton Dickinson).

To study a dose-dependent effect of rebamipide on antigen presentation, the B cells were incubated for 30 min at 37°C with HSA 64-76 peptide-containing medium (25 μM) in order to load the APC with the antigen. Then, the HSA peptide-specific T cells were added together with rebamipide applied at final concentrations of 0.01, 0.05, 0.1, 0.5, 1, and 2 mM. After 24 h of incubation at 37°C under mild agitation, the supernatants were collected and T-cell activation was assessed by measurement of interleukin-2 (IL-2) secretion using an enzyme-linked immunosorbent assay (anti-mouse IL-2 [Duoset]; R&D Systems).

In order to test a potential toxicity of rebamipide with respect to B and T cells, a study of cell mortality using a trypan blue staining and a study of cell apoptosis using flow cytometry (TACS Annexin V-FITC Apoptosis Detection Kit; R&D Systems Europe) were performed. The possible effect of dimethyl sulfoxide (rebamipide solvent) on the system was tested in parallel.

In vitro absorption of rebamipide across the epithelial barrier. Rebamipide has mainly a topical action on gastrointestinal mucosa with poor systemic absorption. However, in order to get in contact with the mucosal immune cells, the drug has to be able to cross the digestive mucosa at a concentration sufficient to interfere with the immune system. Accordingly, using the human intestinal epithelial HT29-19A cells grown as monolayers as an in vitro model of digestive epithelium, we tested whether rebamipide could be absorbed across the digestive epithelial barrier.

The filter-grown HT29-19A monolayers were mounted in small Ussing chambers, as described previously (30). Electrical parameters, PD, and R of the tissue were measured, and ^{3}H-rebamipide (specific activity = 2.44 Ci/mmol) was introduced into the apical (mucosal) compartment of the Ussing chambers at the final concentration of 2 mM. The concentration of rebamipide on the opposite side was evaluated by sampling (500 μl) the basal compartment at 30-min intervals and measuring the concentration of radiolabeled rebamipide by β-scintillation counting. Unidirectional apical-to-basal fluxes of rebamipide (JReb) were calculated using the following equation: $\mathrm{JReb} = \Delta Q/dt \cdot 1/A$, where ΔQ represents the amount of rebamipide accumulated in the basal compartment during the time interval dt and A represents the exposed area of tissue. Fluxes are expressed in microgram-hours per centimeter squared.

Statistical analysis. Statistical analysis was performed using the SAS package. The results are expressed as mean ± standard deviation (SD), and comparison of different parameters among the groups was performed by using analysis of variance and nonparametric tests (Wilcoxon). The differences were considered significant for a P of <0.05.

RESULTS

Development of oral tolerance to OVA in control- and *H. felis*-infected mice. (i) Electrical parameters of gastric and intestinal mucosa studied in Ussing chambers. At the gastric level, there was no significant difference in the electrical parameters, PD and R, among the groups. However, at the intestinal level, the rebamipide-treated mice (group IV) presented a significantly higher R ($56.9 \pm 15.4 \Omega \cdot cm^2$) than the mice from all other groups ($43.0 \pm 15,4$, 38.0 ± 15.9, and $40.4 \pm 16.4\ \Omega \cdot cm^2$, for groups I, II, and III, respectively) ($P < 0.01$). These results suggest that rebamipide reinforces integrity of the intestinal barrier.

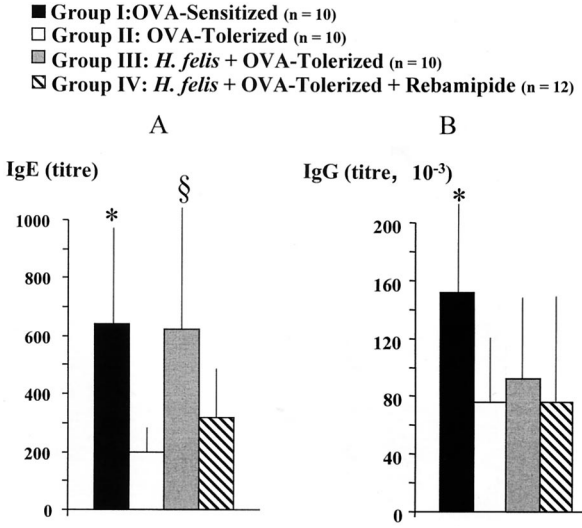

FIG. 1. Specific anti-OVA serum IgE (A) and IgG (B) titers (mean ± SD [error bars]). OVA-sensitized mice present significantly higher IgE and IgG titers than OVA-tolerized mice and mice treated with rebamipide. *H. felis*-infected OVA-tolerized mice present significantly higher IgE titers than OVA-tolerized mice, reflecting the inhibition of the development of oral tolerance by *H. felis*. Symbols: *, significantly different from groups II ($P < 0.03$) and IV ($P < 0.04$); §, significantly different from groups II ($P < 0.01$) and IV ($P < 0.05$); *n*, number of mice studied.

(ii) OVA-specific IgE and IgG antibodies. (a) IgE. OVA-sensitized mice (group I) presented significantly higher serum anti-OVA IgE titers (640 ± 471) than OVA-tolerized mice (group II, 196 ± 104, $P < 0.03$) (Fig. 1A). In *H. felis*-infected OVA-tolerized mice (group III), we observed significantly higher IgE titers ($P < 0.04$) than in noninfected OVA-tolerized mice (group II), suggesting that *H. felis* infection inhibited the development of oral tolerance to OVA. In addition, the mice treated with rebamipide (group IV) presented lower IgE titers than those observed in OVA-sensitized mice (group I) and *H. felis*-infected OVA-tolerized mice (group III), showing that rebamipide can prevent the inhibition of oral tolerance by *H. felis.*

(b) IgG. OVA-sensitized mice (group I) presented significantly higher specific anti-OVA IgG titers ($152 \times 10^3 \pm 84 \times 10^3$) than the OVA-tolerized mice ($76.5 \times 10^3 \pm 47.3 \times 10^3$, $P < 0.03$) (Fig. 1B).

(c) IgG1 and IgG2a. OVA-sensitized mice (group I) presented significantly higher specific anti-OVA IgG1 titers ($191 \times 10^3 \pm 165 \times 10^3$) than the OVA-tolerized mice ($66.6 \times 10^3 \pm 82.3 \times 10^3$, $P < 0.01$) and mice treated with rebamipide ($40.9 \times 10^3 \pm 26.4 \times 10^3$, $P < 0.002$) (Fig. 2). *H. felis*-infected, OVA-tolerized mice presented anti-OVA IgG1 titers ($117.4 \times 10^3 \pm 94.2 \times 10^3$) not significantly different from those obtained in the three other groups. IgG2a titers were similar in all mice (group I, $5.4 \times 10^3 \pm 7.1 \times 10^3$; group II, $6.0 \times 10^3 \pm 6.4 \times 10^3$; group III, $5.6 \times 10^3 \pm 7.0 \times 10^3$; group IV, $4.7 \times 10^3 \pm 6.0 \times 10^3$).

These results suggest that infection with *H. felis* can inhibit the development of oral tolerance to OVA. This inhibition concerns mainly IgE production (Th2 response) and can be prevented by rebamipide.

(iii) Measurement of gastric and intestinal anaphylaxis to serosally applied OVA in Ussing chambers. Despite the high IgE and IgG titers at the systemic level, there was no intestinal or gastric anaphylactic response noted in any of the mice (data not shown). This suggests that in this model, reaginic antibodies may not be present locally in the digestive mucosa.

Development of sensitization to orally administered HEL+CT in control- and *H. felis*-infected mice. (i) Electrical parameters of gastric and intestinal mucosa studied in Ussing chambers. At the gastric level, there was no significant difference in the electrical parameters among the groups. At the intestinal level, the mice treated with rebamipide (group IV) presented *R* values significantly higher ($47.2 \pm 13.2\ \Omega \cdot cm^2$) than those of the mice from groups II ($36.5 \pm 13.4\ \Omega \cdot cm^2$; $P < 0.02$) and III ($37.05 \pm 15.4\ \Omega \cdot cm^2$; $P < 0.04$). These results confirm that rebamipide enhances the epithelial intestinal integrity in mice.

(ii) HEL-specific IgE and IgG antibodies. (a) IgE. As expected, mice sensitized to HEL (group II) presented significantly higher anti-HEL IgE titers in serum (448 ± 335) than the control mice (group I) (135 ± 118, $P < 0.004$) (Fig. 3A). In *H. felis*-infected and HEL-sensitized mice (group III), anti-HEL IgE titers were similar to those found in noninfected, sensitized mice. Mice treated with rebamipide (group IV) presented IgE titers similar to those found in control mice (174 ± 99) and significantly lower from those found in groups II ($P < 0.002$) and III ($P < 0.04$).

(b) IgG. The anti-HEL IgG titers showed a pattern similar to that of IgE titers, with high IgG titers in groups II ($144 \times 10^3 \pm 80 \times 10^3$) and III ($182 \times 10^3 \pm 17 \times 10^3$), which were significantly higher than those found in control mice ($1.2 \times 10^3 \pm 0.4 \times 10^3$) ($P < 0.004$ and 0.03, respectively) and nonsig-

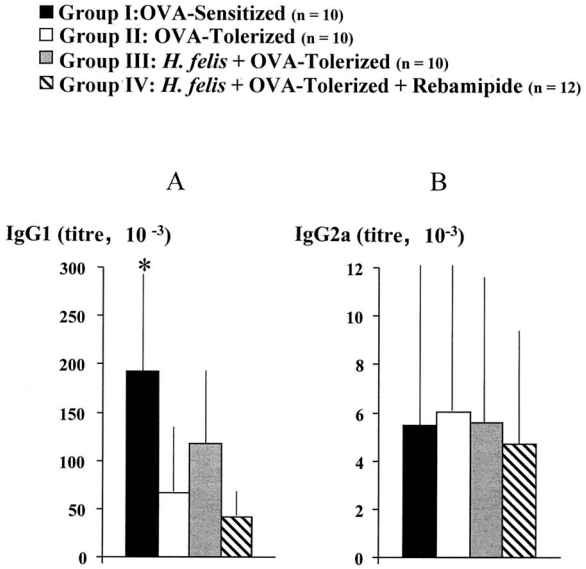

FIG. 2. Specific anti-OVA serum IgG1 (A) and IgG2a (B) titers (mean + SD [error bars]). OVA-sensitized mice present significantly higher IgG1 titers than OVA-tolerized mice or mice treated with rebamipide. *H. felis*-infected and OVA-tolerized mice present nonsignificantly different IgG1 titers from those presented by noninfected OVA-tolerized mice. No significant differences are found in IgG2a titers among the groups. *, significantly different from groups II ($P < 0.01$) and IV ($P < 0.002$); *n*, number of mice studied.

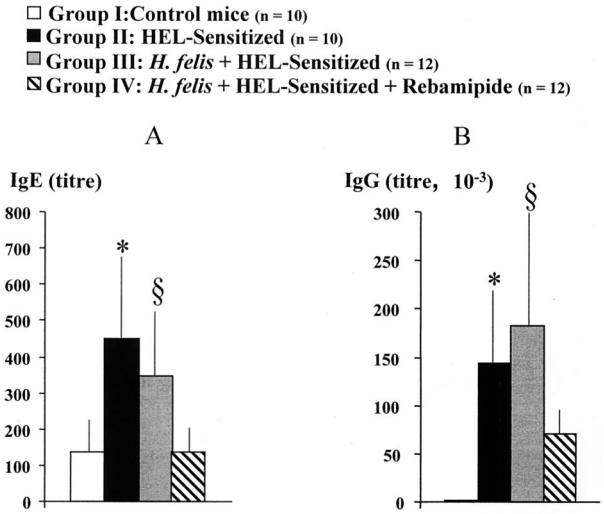

FIG. 3. Specific anti-HEL IgE (A) and IgG (B) titers (mean + SD [error bars]) in sera from mice. (A) Symbols: *, significantly different from groups I ($P < 0.004$) and IV ($P < 0.002$); §, significantly different from groups I ($P < 0.04$) and IV ($P < 0.04$). (B) Symbols and abbreviations: *, significantly different from group I ($P < 0.03$); §, significantly different from group I ($P < 0.004$); *n*, number of mice studied.

nificantly higher from those found in rebamipide-treated mice (group IV) ($70 \times 10^3 \pm 35 \times 10^3$) (Fig. 3B).

These results suggest that *H. felis* does not influence significantly the development of sensitization to HEL in this model and that rebamipide can inhibit the sensitization process.

(iii) Measurement of gastric and intestinal anaphylaxis to serosally applied HEL in Ussing chambers. As observed in OVA-sensitized mice, the presence of systemic antibodies to HEL did not correlate with a local anaphylactic response in the presence of HEL (data not shown).

Effect of rebamipide on antigen presentation in vitro. As shown on Fig. 4, there was a dose-dependent inhibitory effect

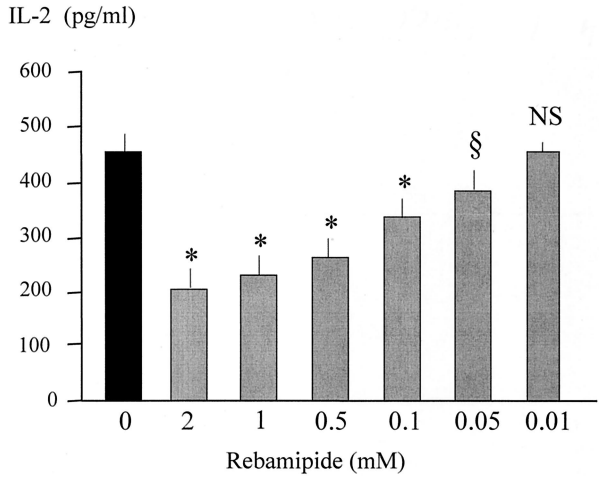

FIG. 4. Dose-dependent inhibitory effect of rebamipide on T-cell activation (IL-2 secretion) in an in vitro model of antigen presentation (mean + SD [error bars]; $n = 8$). Symbols and abbreviations: * and §, significantly different from controls (*, $P < 0001$; §, $P < 0.004$); NS, nonsignificant difference; *n*, number of experiments for each set of conditions (concentration).

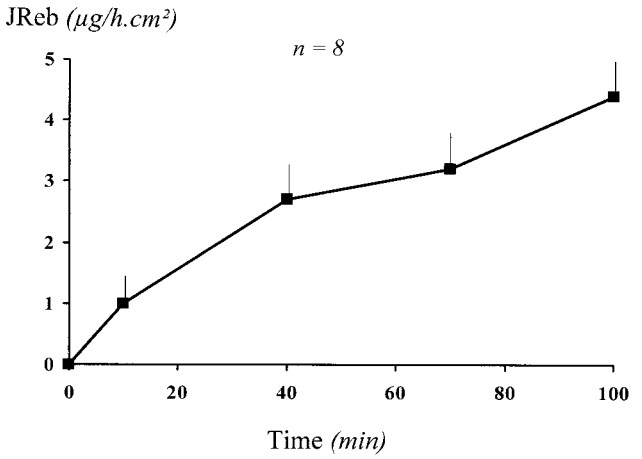

FIG. 5. Apical-to-basal rebamipide fluxes (JReb) across HT29-19A intestinal monolayers mounted in Ussing chambers (mean + SD [error bars]). *n* = number of measurements for each time point.

of rebamipide on T-cell activation in this in vitro model of antigen presentation. Importantly, at a concentration as low as 0.05 mM, rebamipide still revealed its inhibitory effect.

Analysis of cell mortality by trypan blue and of apoptosis of B and T cells showed no difference in cell mortality between rebamipide-treated (2 mM for 24 h) and control cells.

No effect of dimethyl sulfoxide on the system in the corresponding concentrations was observed (not shown).

In vitro rebamipide absorption across the digestive cell monolayer in Ussing chambers. The apical-to-basal fluxes of rebamipide across filter-grown HT-29 monolayers are presented on Fig. 5. A gradual increase in rebamipide fluxes which reached the value of 6.6 ± 0.7 μg/h·cm^2 (17 μmol/h·cm^2) after 100 min was observed, indicating that rebamipide is capable of crossing the epithelium.

DISCUSSION

This study shows that, in mice, infection with *H. felis* can prevent the development of oral tolerance to OVA. However, *H. felis* infection does not enhance the sensitization to oral HEL obtained in the presence of CT, suggesting that bacterial infection does not augment the adjuvanticity of CT. In addition, a mucosal stabilizer, rebamipide, is able to prevent the deleterious effect of *H. felis* on oral tolerance and to attenuate the oral sensitization to HEL.

The mucosal immune system generally induces local and systemic tolerance to dietary antigens. However, enteric pathogens and their products may play a critical role in disrupting this nonresponsiveness. CT (13) and *E. coli*-heat labile enterotoxin (18) can inhibit oral tolerance in mice. Only few data are reported on the possible impact of chronic digestive infections on the development of oral tolerance to dietary antigens. A helminth infection was shown to inhibit the oral tolerance to OVA for the Th2-dependent IgG isotype (IgG1) (33). Similarly, intestinal inflammation, associated with an increased gut permeability, led to the enhancement of sensitization to ingested milk proteins in guinea pigs (15). Our results indicate that chronic infection with *H. felis* inhibits the establishment of oral tolerance by preventing IgE suppression, normally in-

duced after OVA feeding. As IgG1 and IgE antibody responses are regulated by Th2 populations (21), it is likely that a down-regulation of Th2-dependent responses occurred after OVA feeding.

The mechanisms by which the development of oral tolerance is inhibited in the presence of *H. felis* infection may involve the increase in antigenic absorption across the epithelium (28, 29) and the presence of the bacterial adjuvants. *H. pylori* can play the role of an adjuvant by inducing an overexpression of proinflammatory cytokines in the mucosa (14) and costimulatory molecules (38). Interestingly, the presence of *H. felis* did not influence the development of sensitization to the orally administered dietary antigen HEL. This finding is probably due to the fact that in this model of oral sensitization, HEL is administered together with CT, a strong adjuvant of mucosal immune response, and that the presence of an additional adjuvant factor, *H. felis* infection, did not add to the powerful effect of CT.

The inhibitory effect of *Helicobacter* infection on oral tolerance could contribute to the persistence of chronic gastric inflammation and development of allergic sensitization. Indeed, the association between *H. pylori* infection and food allergy (8, 16) as well as with other allergic diseases like chronic urticaria (12, 35), atopic dermatitis (31), and hereditary angioneurotic edema was suggested, although contradictory results have been obtained (24, 26). The main factor which argues against this association is the Th1/Th2 paradigm. *H. pylori* infection is associated with a Th1 immune response, while in allergic disorders, as well as in parasitic (helminth) infections, a Th2 response predominates. Indeed Th2-dependent helminth preinfection with *H. polygyrus* was shown to attenuate gastric inflammation and gastric epithelial lesions in mice subsequently infected with *H. felis* (17). Theoretically, the Th1 response should protect against the development of allergic diseases, since Th1 and Th2 responses are considered mutually exclusive. This argument is used to explain epidemiological data indicating a low prevalence of allergic disorders in developing countries presenting a high incidence of bacterial infections, including *H. pylori* infection. In contrast, an increasing prevalence of allergic disorders occurs in Western countries where the incidence of bacterial infections is low. For a long time it has been proposed that bacterial infections occurring early in life direct the maturing immune system toward a Th1 component, which counterbalances the proallergic Th2 responses. However, this hypothesis is now being contradicted by observations that Th1 autoimmune diseases (i.e., type 1 diabetes) are also increasing and that helminth infections are inversely correlated with allergy (25, 37). It is today proposed that it is rather the intensity and quality of a regulatory T-cell network ($CD4^+$ $CD25^+$), induced by persistent immune challenge, which determine the type of immune response which will develop in response to different microbials and allergens, without any exclusion between Th1 and Th2 responses (37). Indeed, epidemiological studies show that Th1- and Th2-type diseases can coexist (22), which indicates a common denominator behind the disease processes and refutes the mutual exclusion of these two types of response. Moreover, as far as oral tolerance is concerned, it has been shown that multiple nonexclusive T-cell-mediated mechanisms can be involved in immune response to food antigens and that both subsets of Th cells are capable of participating in this response (3). Finally, it is possible that in the case of *H. pylori* infection, the allergic sensitization is raised during the initial phase of infection, when the local Th1 response is not yet fully developed.

The present study shows the protective effect of rebamipide with respect to oral tolerance and its capacity to inhibit the development of allergic sensitization to HEL and CT. The effect of rebamipide may be partly explained by its known mucosa-stabilizing and anti-inflammatory properties (6). The beneficial effect of rebamipide on the intestinal epithelial barrier function could play a role. Indeed, rebamipide has been shown to reinforce the epithelial barrier, under both normal and inflammatory conditions in vitro (30), and to favor the normalization of protein transport across the gastric mucosa, increased by *H. felis* infection in mice (27). Moreover, the present study shows also its capacity to reinforce integrity of the intestinal barrier, a phenomenon which may have contributed to the inhibition of the immune response to orally administered antigens in infected animals. Rebamipide may have attenuated the immune response to orally administered antigens by inhibiting the secretion of cytokines implicated in antibody production. Indeed, the drug has been shown to inhibit the secretion of IL-1β, IL-8, IL-10, gamma interferon, and tumor necrosis factor alpha by *H. pylori*-stimulated human peripheral blood mononuclear cells or of IL-8 by the gastric epithelial cells (1) and to block the NF-κB pathway (23). It has to be stressed that the blocking of proinflammatory cytokine such as IL-1 by rebamipide may be important since the adjuvancy effect of CT can be reversed by anti-IL-1 treatment (4). The down-regulation of proinflammatory cytokine production by rebamipide may also interfere with antigen presentation by decreasing the expression of costimulatory molecules on APC. Indeed, our results show that rebamipide is capable, at least in vitro, of inhibiting antigen presentation and T-cell activation. Although these results cannot be extrapolated to humans, they suggest the potential ability of rebamipide to inhibit T-cell activation. This inhibition takes place at a concentration as low as 0.05 mM, a concentration corresponding to the rebamipide concentration found within the gastric mucosa after oral ingestion of a clinical dose in healthy volunteers (2). Moreover, according to our results on transepithelial absorption of rebamipide, this concentration is likely to be achieved on the basal side after apical-to-basal rebamipide transfer across the epithelial layer in vitro. It is therefore conceivable that the small quantity of rebamipide absorbed by the digestive mucosa may interfere with the mucosal immune system also in vivo. All our results suggest that rebamipide might be a good candidate for the treatment of posteradication gastritis.

In conclusion, this study shows that gastric infection with *Helicobacter* can inhibit the development of oral tolerance to dietary antigens. The finding that a chronic bacterial infection can increase the immunogenicity of orally absorbed dietary antigens may explain some of the allergic manifestations associated with *H. pylori* infection in susceptible individuals. It is further noticeable that in this animal model, the mucosa-stabilizing agent, rebamipide, is capable of counteracting the deleterious effect of *Helicobacter* on the development of oral tolerance.

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